

APPENDIX I

PROCEDURE FOR THE DISTILLATION OF NICOTINE

1. The following equipment is needed:
 - 1.1 Heated three-neck round bottom flask - 250 mL - Ace Glass Company, Catalog #9642-112.
 - 1.2 Controller for heated flask (above) - Ace Glass Company, Catalog #9698-15.
 - 1.3 Connecting cord for heated flask - Ace Glass Company, Catalog #9698-15.
 - 1.4 Distilling receiver - Ace Glass Company, Catalog #9400-10.
 - 1.5 Distilling column - Ace Glass Company, Catalog #9351-06.
 - 1.6 Condenser, West type, 120 mm long Ace Glass Co. Catalog #9299-08.
 - 1.7 Magnetic stirrer - Fisher Catalogue #14-493-120 MR.
 - 1.8 Adapter (2) - Ace Glass Company, Catalog #5028-28.
 - 1.9 Thermometer (0-250°C) - Ace Glass Company, Catalog #9553-04.
 - 1.10 Thermometer (-10 to + 250°C) - Ace Glass Company, Catalog #8285-16.
 - 1.11 Vacuum trap (2) - Ace Glass Company, Catalog #8753-12.
 - 1.12 DeWaar Flask (2) - Ace Glass Company, Catalog #2540-07.
 - 1.13 Needle valve (bottom of a Fisher burner) - Fisher, Catalog #03-902.
 - 1.14 Manometer - McLeod (5 um to 5 mm) - Fisher, Catalog #10-269-27.
 - 1.15 Teflon sleeves - Fisher, Catalog #14-320D.
 - 1.16 Vacuum pump - Welch from Fisher, Catalog #01-096.
 - 1.17 T-Shaped Connectors, Glass, 5/10" O.D. - Fisher Scientific, Catalog #15-328B.
 - 1.18 Storage vials, 1 mL capacity - Preiser, Catalog #10-4788-12.
 - 1.19 Caps for 1 mL vials - Preiser, Catalog #10-4805-29.
 - 1.20 Crimper for above caps - Preiser, Catalog #10-4814-13.

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- 1.21 Large storage vials with caps, 50 mL capacity - Preiser, Catalog #72-4708-53.
 - 1.22 Transfer syringe, 2 mL capacity - Preiser, Catalog #14-4249-17.
 - 1.23 Needles for above, No. 18, 2" - Preiser, Catalog #14-4261-52.
 - 1.24 Regulator, Dual Stage Brass, for nitrogen - Matheson, Catalog 3104-580.
 - 1.25 Needle valve - for regulator above - Nupro, Catalog-Ss-2-25
2. Supplies
- 2.1 Nicotine, Reagent Grade, 98% purity - Eastman Organic Chemicals, Catalog #1242.
 - 2.2 Vacuum grease, Apiezon T - Fisher Scientific Company, Catalog #14-638-15E.
 - 2.3 Rubber tubing, $\frac{1}{2}$ " base x $\frac{1}{4}$ " wall - Preiser Scientific, Catalog #13-8874-13.
 - 2.4 Magnetic stirring bar (1"), Teflon Coated - Fisher Scientific, Catalog #14-511-63.
 - 2.5 Nitrogen, gas cylinder - Prepurified Grade from Matheson.
3. Safety Requirements
- NOTE: NICOTINE IS A POISON AND SHOULD BE HANDLED WITH CARE!
- 3.1 This equipment should be set up in a well ventilated hood that has a glass safety shield.
 - 3.2 There should be two technicians present at all times when the distillation is in process.
 - 3.3 Technicians must wear safety glasses with side shields or full face safety shields.
 - 3.4 Technicians must wear rubber gloves when handling nicotine.

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4. Equipment Set-up

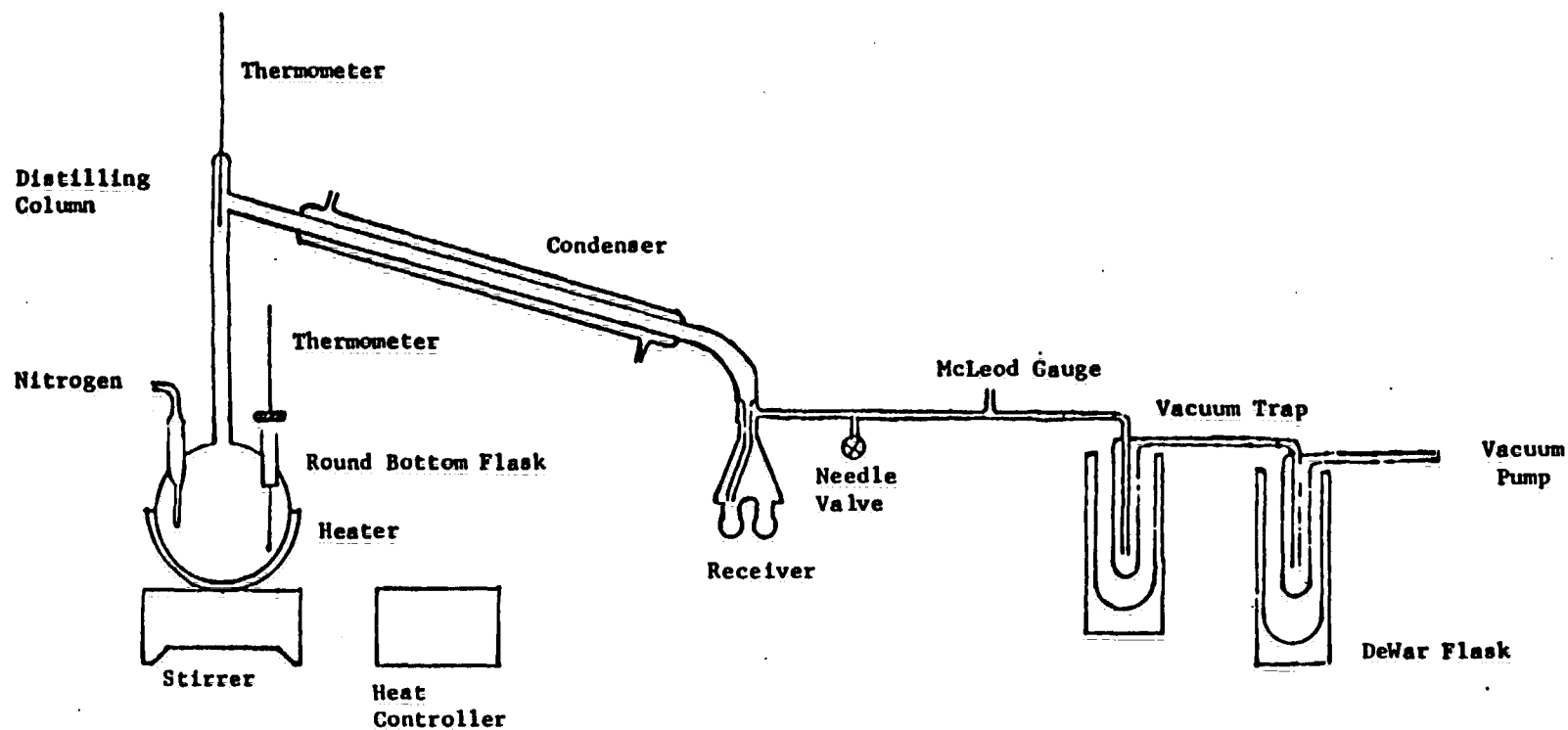
- 4.1 All glassware should be cleaned in a chromic acid solution, rinsed in distilled water and dried in an oven before use.
- 4.2 Assemble equipment as shown in Figure 1 using the Teflon sleeves to minimize the use of vacuum grease.
- 4.3 Test system for leaks by closing needle valve on nitrogen inlet, starting vacuum pump and gradually closing the system needle valve. It is not necessary to have dry ice/acetone mixture in the DeWar flasks.
- 4.4 Using the McLeod gauge, the vacuum should be less than 0.5 mm Hg.

5. Distillation Procedure

- 5.1 Carefully add the contents of a 100 g bottle of nicotine to the distillation flask.
- 5.2 Add a stirring bar and start the stirring motor.
- 5.3 Purge the system with N_2 and adjust flow to about 1-2 mL/min.
NOTE: Keep gas delivery tube ABOVE the surface of the liquid.
- 5.4 Add dry ice and acetone to the two vacuum traps in the DeWar flasks.
- 5.5 Make sure that the system needle valve is open and turn on the cooling water to condenser.
- 5.6 Start the vacuum pump and gradually close the system needle valve.
- 5.7 Check the vacuum frequently with the McLeod gauge.
- 5.8 When the vacuum stabilizes, adjust the vacuum level to 1 mm Hg by adjusting the flow of the nitrogen purge.
- 5.9 Gradually apply heat to the distillation flask.
- 5.10 Distillation will start at about 90°C. Distill at the rate of one drop every 15 seconds.
- 5.11 Depending on the pressure, nicotine will distill at 110-120°C.
- 5.12 Collect fractions and label with boiling range and pressure.
- 5.13 The residue remaining in the distilling flask should be weighed and transferred to an amber vial. A label should be placed on the vial stating nicotine residue and the weight. The vial should be returned to the Stockroom for disposal.

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FIGURE 1
EQUIPMENT SET-UP FOR NICOTINE DISTILLATION



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6. Storage

- 6.1 After distillation, transfer the distilled nicotine using a glass syringe with a No. 18 or 20 needle to septum vials.
- 6.2 Purge the vials with nitrogen and close with a Teflon lined cap.
- 6.3 Store three or four vials in a larger screw cap vial that has been purged with nitrogen.
- 6.4 Carefully label showing date, temperature range, pressure, and person carrying out distillation.
- 6.5 Wrap the container in aluminum foil and store in the dark in a freezer.
- 6.6 The the samples will remain colorless for about six months if stored under these conditions.

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